SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #6

July 31, 1996

The University of Michigan

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Quarterly Progress to:	National Institute of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract	"Surface Modification for Biocompatibility"
Contract No.	NS 5-2322
Principal Investigators:	David C. Martin and K. Sue O'Shea
Date:	July 31, 1996

Overview

This report is a summary of our activity in the sixth quarter of the contract, corresponding to the second quarter of 1996. In this sixth period of our activity we have continued to refine our processing and characterization schemes. We have also obtained information about the biological response of the protein coated suture during *in vivo* implantations. This report provides an overview of the major results to date and discusses our plans for the future. We have been working to evaluate (1) protein polymer film deposition and morphology, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition, Morphology, and Device Characterization

Progress:

In this quarter we have confirmed the versatility of our processing schemes for creating protein polymer coatings which can be blended with a number of different small molecules. We anticipate that the ability to create polymer coatings which can be loaded with secondary components will prove to be useful in the optimization of our materials and their biological activity.

We have now shown that a number of different agents can be coprocessed with the protein polymer to create porous coatings with a controlled microstructure. Our experiments have recently focused on establishing the variations in morphology observed with agents of differing solubilities in the formic acid solvent usually used for protein polymer solution processing. To this end, we have examined aspirin, which is soluble in formic, and caffeine, which is not. To facilitate our process development, we have also examined synthetic polyamide polymers as carriers for the small molecules. Rheological characterizations have revealed that the manner in which the viscocity of the aliphatic polyamide varies with concentration is similar to that of the protein polymer, so that it provides a useful means to develop processing schemes and equipment without using the protein polymer, which is only available in limited quantities.

Our past reseach has established that electric fields can be used to control the deposition of polymers onto surfaces of solid substrates. We have now shown that this approach can also be easily generalized to examine polymer blends with other materials. For a 150 mg/ml solution of aliphatic polyamide in formic acid, the microstructure of the deposited film consists of uniform filaments on the solid surface. This fibrous morphology is maintained for additions of aspirin up to 50% by weight in the solution (Figure 1). By

56% the fiber morphology becomes difficult to resolve, and variations in thickness along the filament length are evident. At higher concentrations, the fibrous morphology is no longer observed. TEM experiments indicate that the mixing of the polymer and aspirin is initimate, in that lamellar crystals of the polymer can be observed essentially uniformly distributed throughout the sample.

SEM images of caffeine/aliphatic polyamide blends shows that the concentration at which significant variations in filament diameter begin is lower in this case (20%) (Figure 2) . These changes correlate with the reduced solubility of the caffeine in the formic acid solvent. We also find that the small molecules can be readily processed in a similar manner with the protein polymer as the matrix. Figure 3 shows a film containing 25% aspirin in the fibronectin-modified SLPF polymer.

Now that the flexibility and convenience of our approach has been established, we have acquired a number of antibiotics for further evaluations including tetracycline, cephalosporin, bacitracin, amoxicillin, hygromycin, neomycin, kanamycin, erythromycin, and nystatin. Compounds of specific interest to our future work include growth factors such as NGF and GDNF, anti-mitotics, anti-inflammatory agents, and chemoattractants. We are actively working with our colleagues in the Kresge Hearing Research Institute to identify agents which are likely to be of the most utility for these coatings, and would be happy to solicit specific suggestions for other compounds of interest to investigators, since it appears that our approach is fairly flexible and versatile. Of concern will be the solubility of the material in formic acid, and the rate at which this compound should be released for a given application. We suspect that the kinetics of release will be initmately related to the size of the protein filaments produced and the thickness of the deposited film.

We have quantified the surface structure of our films by examining the power spectral density (PSD) of scans obtained by atomic force microscopy (AFM). The PSD makes it possible to examine the evolution of surface roughness as a function of length scale. At the biologically relevant length scales > 1.0 micron, we find that the roughness increases with coating thickness, However, at the smaller lengths scales, the films become smoother and more continuous as they thicken. These smaller length scales have implications for film adhsion, mechanical properties, and electronic transport. It therefore appears that a film which has an optimum combination of mechanical integrity, efficient cellular adhesion, and low impedence will be found with an intermediate amount of material which is sufficiently rough and continuous to bind cells, but not interrupt the efficiency of carrier transport across the interface to the iridium/silicon substrate.

Information about the electrical performance of the protein polymer coated probes continues to be obtained by impedance spectroscopy. The typical impedance characteristics of a given electrode site consists of an initially decreasing (capacitive) response of the amplitude of the impedance at frequencies below 10^4 Hz, with a transitition to a frequency-independent (resistive) response at high frequencies. After activation, the impedance is lowered by about a decade in the low frequency range, although the effect seems to be less at higher frequencies (Figure 4). After dipping in formic acid, the probes are also lowered in impedence, although the effect is not as large as activation. On the other hand, the impedance changes in this case are observed across the frequency spectrum (Figure 5). SEM images of the probes before and after the impedance testing have confirmed that the films are stable to the testing conditions employed (Figure 6).

Experiments to evaluate the mechanical properties of the protein films have also continued. We have found that the more continuous films are more brittle than films with a porous or fibrillar morphology.

Plans:

We are initiating experiments to characterize the mechanical properties of the polymer thin films using a thin film mechanical testing device (Nanoindentor) recently acquired and installed in the University of Michigan Electron Microbeam and Analysis Laboratory.

We have also begun a series of experiments to examine the bioactivity and electrical properties of electrodeposited films as a function of thickness. These results will make it possible for us to correlate results from the dip coated films, and make it possible to test the hypothesis that the discontinous films will be most efficient at promoting cell binding, maintaining mechanical integrity, and facilitating electronic transport from the prosthesis to the living tissue of the CNS.

2. Bioactivity of Protein Polymer Films *in vitro*

Progress:

We have begun a series of in vitro experiments to examine the biological efficacy of electrodeposited films as a function of thickness and coating morphology. We also plan to test the films containing various amounts of small molecules with biological activities.

We are continuing to develop coatings with alternating stripes and patches of SLPF (fibronectin) and SLPL (laminin) protein polymers, with the intent of directing cell attachment. Our hypothesis is that the SLPF domains will be preferential toward the attachment of glial cells, while SLPL will be favorable toward neurons. SELP patches could also be used to resist cellular adhesion. Examinations of the variation of cellular adhesion with time will provide information about the dynamics of this process. It will then be possible to imbibe the polymer coatings will small molecules of interest for promoting a specific biological response.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

Polypropylene suture (~50 micron diameter) was coated with the following materials and implanted in the Guinea Pig CNS:

1. no coating (control)
2. SLPF coated
3. SLPL coated
4. SLPF/Schwann cells
5. SLPF coated and exposed to CSF
6. SELP coated

The histological results have now been correlated and quantified. Results were obtained by both optical microscopy, scanning electron microscopy, and transmission electron microscopy.

In the SEM, it is possible to distinguish the interface between the CNS and the PP suture. The asymmetry of the protein film can be noted by examining the thickness of the

coating around the outside of the fiber, due to the unidirectional deposition of the protein onto the fiber. The TEM cross-sections reveal the microstructure of the coated PP suture and CNS in detail. The substructure of the PP suture is evident, including particles which apparently relate to the dyes used to give the suture a visibly blue color. At the surface of the PP, it is possible to locate a dense layer which is consistent with the protein polymer. The structure of the protein film indicates that it has maintained local, intimate contact with the PP substrate, so that adhesion between the polymer and PP does not appear to be a problem. Further, the outer surface of the protein is rougher than the suture surface, consistent with our goal of making a large number of contact sites for promoting cellular adhesion. In the region of the CNS near the SLPF protein there is evidence for an electron dense layer in the unstained samples. Staining reveals this layer to be composed of a layer of cell bodies which have a flattened morphology consistent with a strong adhesive interaction. (Figures 7 - 13).

Discussions of the optical microscopy and TEM data have indicated that the response of the tissue to the implants is "light". The neurons appear to be a somewhat stressed state, but remain alive. The happiness of the neurons nearest the probes might be further improved by neurotrophins or growth factors which could improve their activity.

It is clear that the response of the CNS to the protein polymer coated implanted suture is minimal. In short, there has been no evidence for any dramatic problems associated with the implanted materials. This is in dramatic contrast to the implantation of other components which have been used in past studies, and we believe provides considerable motivation for our future efforts in this area. Per our agreement with Dr. Cappello of Protein Polymer Technologies, Inc., we are preparing a detailed report of our findings, which will be included with our next quarterly report.

Plans:

The nine-month embedded implants will be available for examination in August of this year. The EECS and Kresge group have also been making progress in the development of techniques for probe performance evaluation by confocal microscopy, and we hope to work with them to answer questions of mutual interest.

We have also been in communication with Dr. Allen Mensinger at St. Louis and intend to coat probes for his evaluation during August. The results of his investigations should provide additional information about the *in vivo* performance of these materials.

4. External Communications

The invited review paper on *Processing and Characterization of Silk-like Protein Polymers*, was revised by coauthors David C. Martin, Chris Buchko, and Tao Jiang, which is scheduled to appear in a special volume on *Protein Polymer Materials*, edited by Kevin McGrath and David Kaplan of the U. S. Army Natick RD&E Center, is now under review by the publisher. A copy of this document is enclosed with this report.

An abstract titled "Electrostatic Deposition of Protein Polymer Blends" was submitted by Chris Buchko, Loui C. Chen, Michael A. Johnson, and David C. Martin for the Materials Research Society meeting in December 1996.

An abstract titled "Impedance Spectroscopy of Protein Polymer Coated Micromachined Silicon Probes" was submitted by Shenkarram Athreya, James D. Weiland, David J. Anderson, and David C. Martin for the Materials Research Society meeting in December 1996.

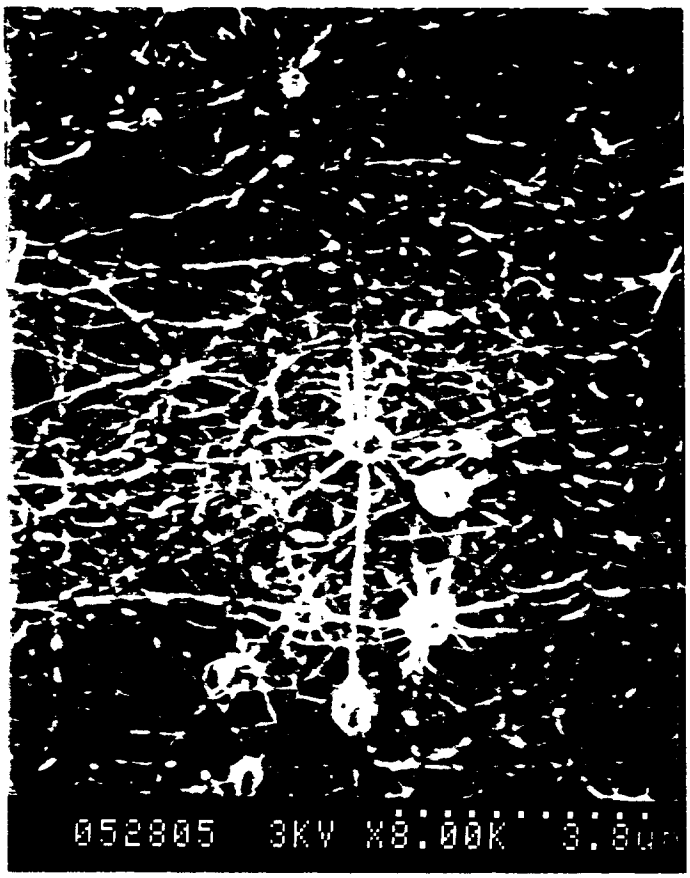


Figure 1

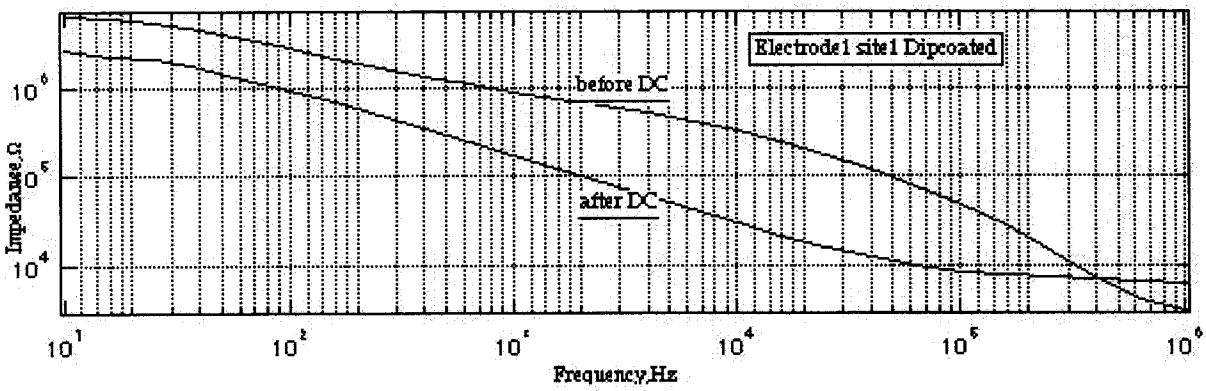
Scanning Electron Micrograph of electrodeposited blend coating of 56% by weight aspirin in aliphatic polyamide on silicon substrate

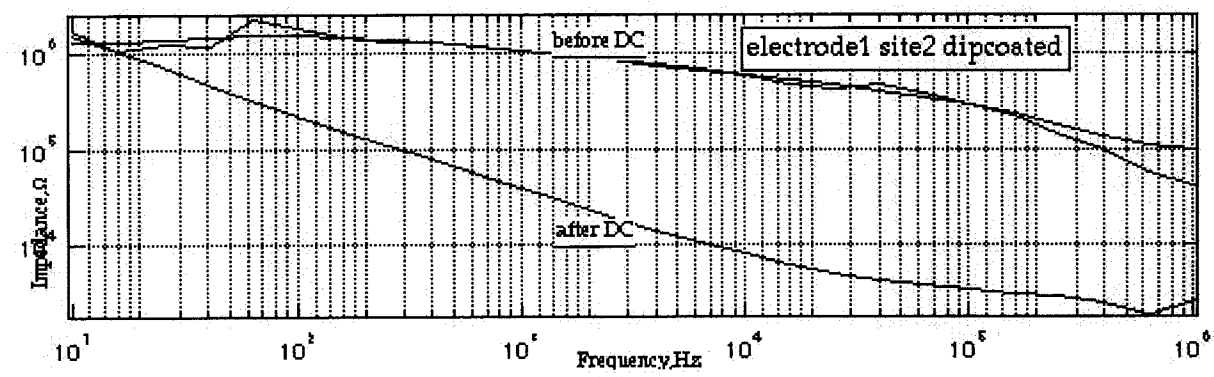
Figure 2

Scanning Electron Micrograph of electrodeposited blend coating of 20% by weight caffeine in aliphatic polyamide on silicon substrate

Figure 3

Scanning Electron Micrograph of electrodeposited blend coating of 25% by weight aspirin in SLPF protein polymer on silicon substrate


Electrode1 site1 Dipcoated
before DC
after DC
Impedance,Ω
Frequency,Hz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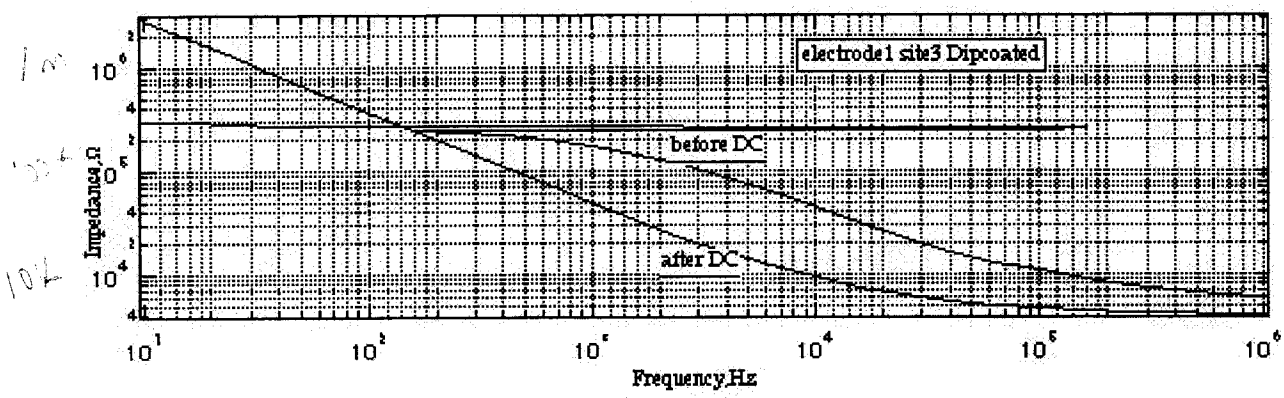

electrode1 site3 Dipcoated
before DC
after DC
Impedance,Ω
Frequency,Hz

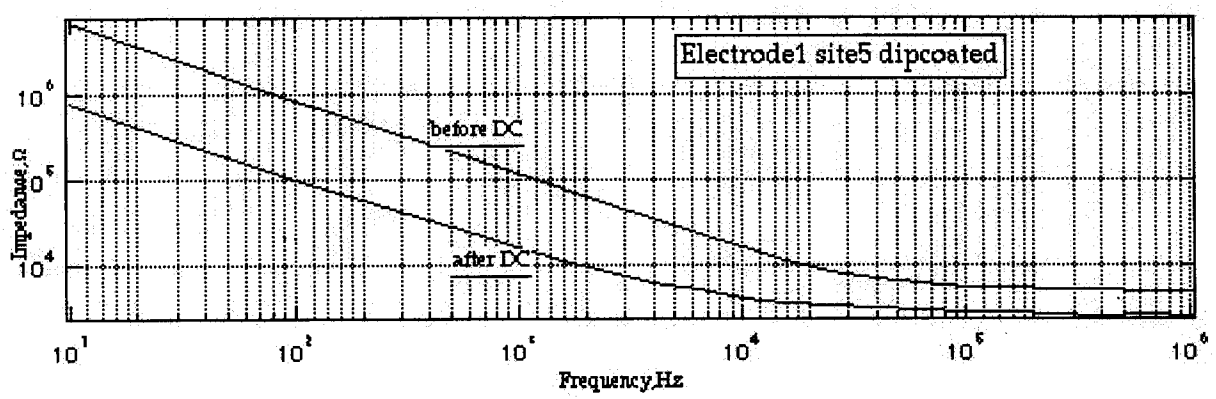
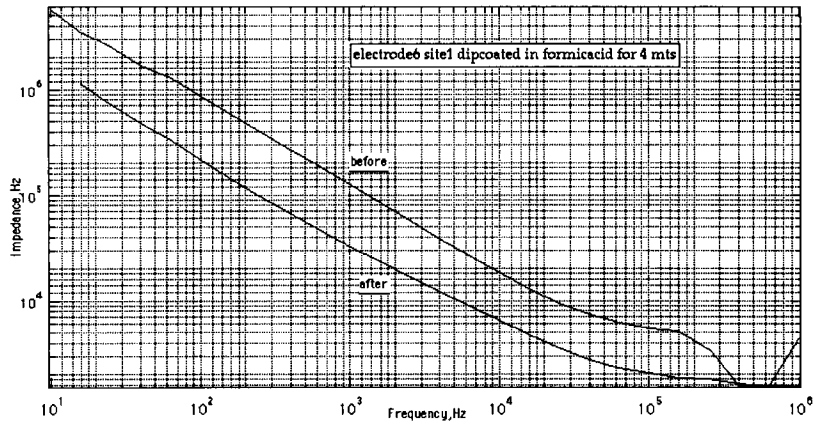
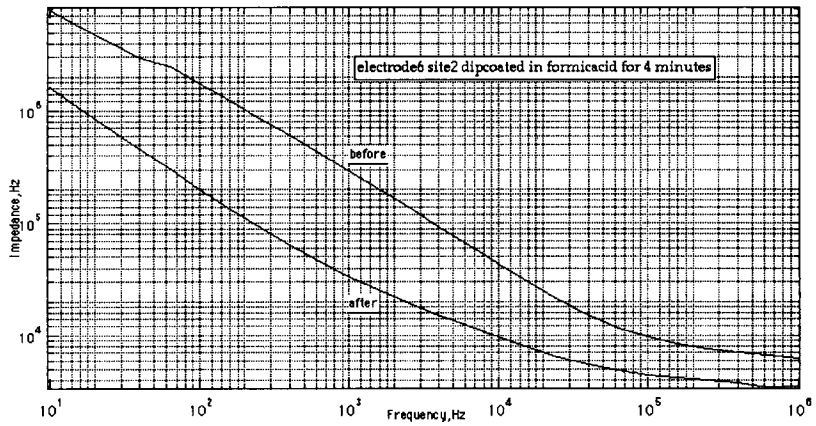
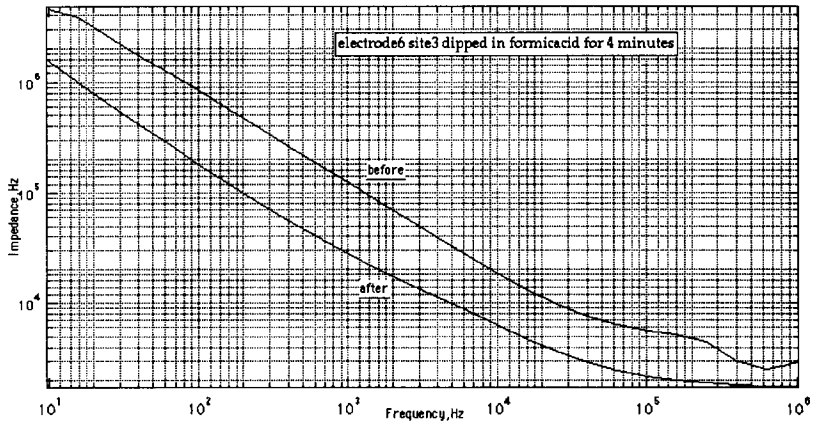

Electrode1 site5 dipcoated
before DC
after DC
Impedance,Ω
Frequency,Hz

Figure 4


electrode6 site1 dipcoated in formicacid for 4 mts
before
after
Impedance,Hz
Frequency,Hz


electrode6 site2 dipcoated in formicacid for 4 minutes
before
after
Impedance,Hz
Frequency,Hz


electrode6 site3 dipped in formicacid for 4 minutes
before
after
Impedance,Hz
Frequency,Hz

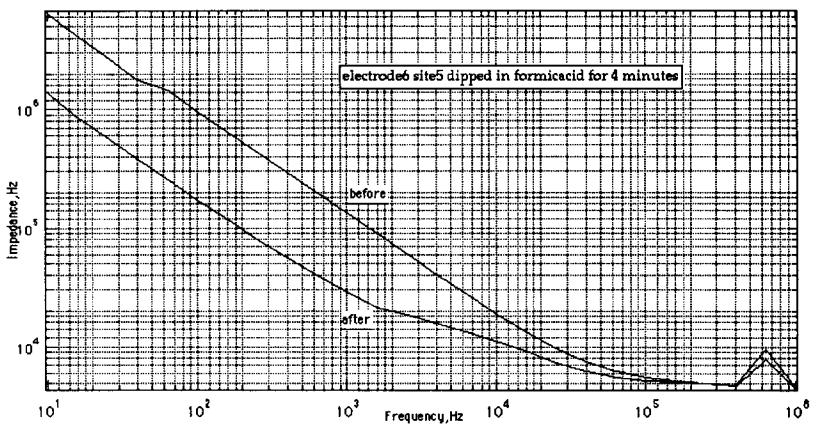
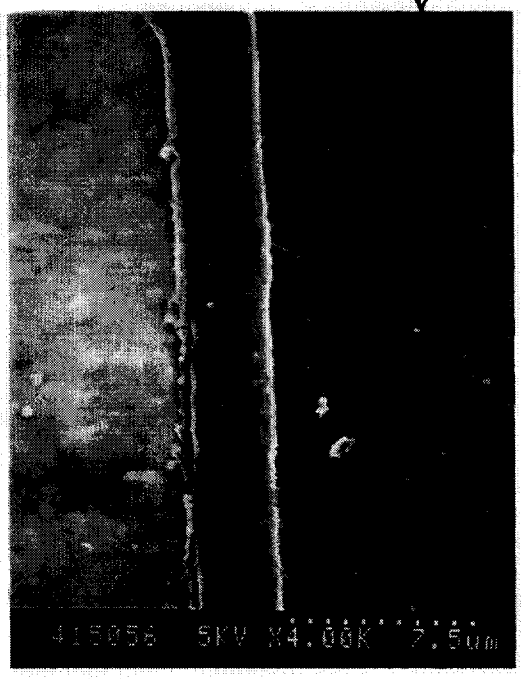

electrode6 site5 dipped in formicacid for 4 minutes
before
after
Impedance,Hz
Frequency,Hz

Figure 5

protein polymer filaments deposited on surface



Iridium Pad

polysilicon

silicon

SEM of protein polymer coated probe

Figure 6

Figure 7

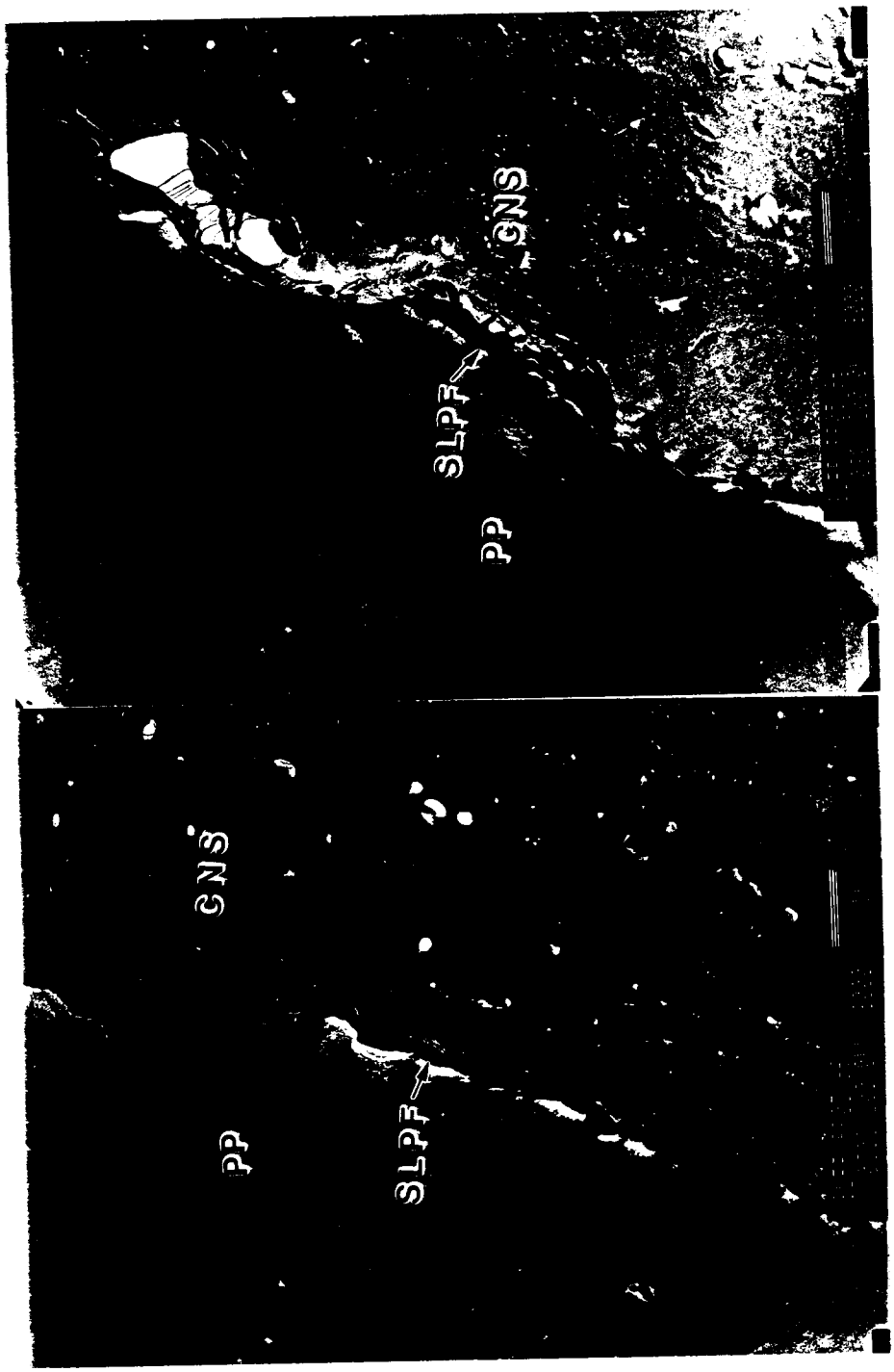
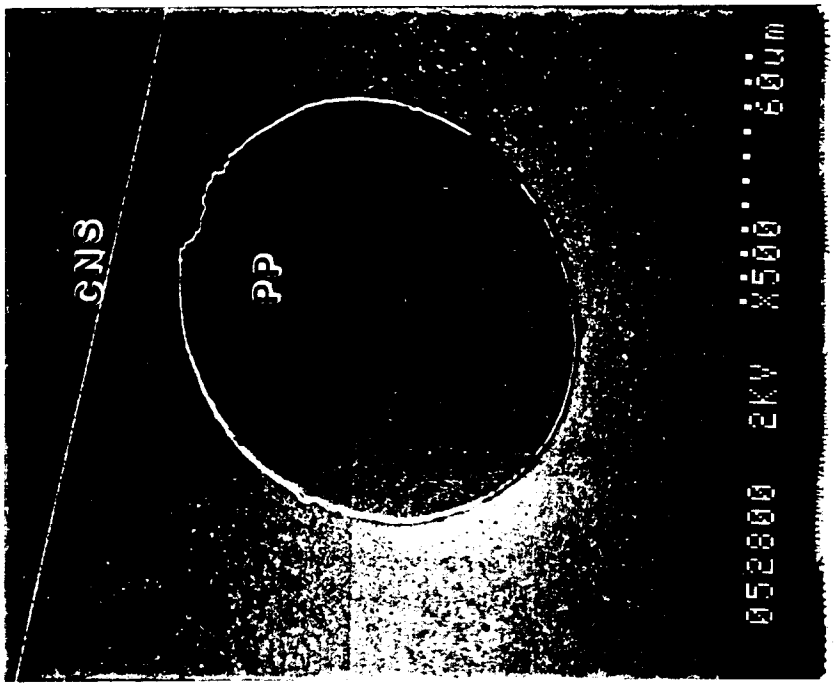


Figure 8



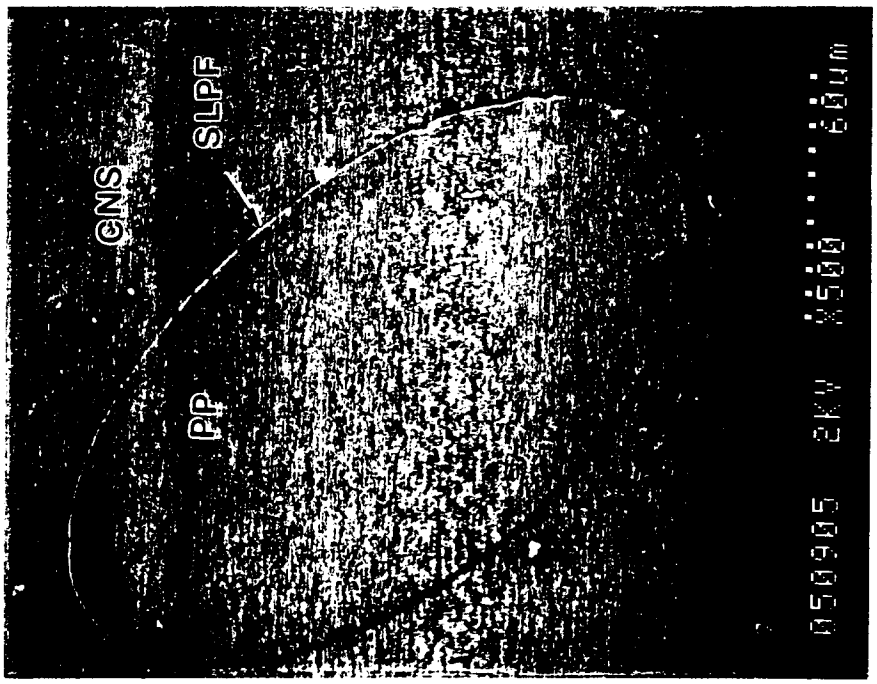
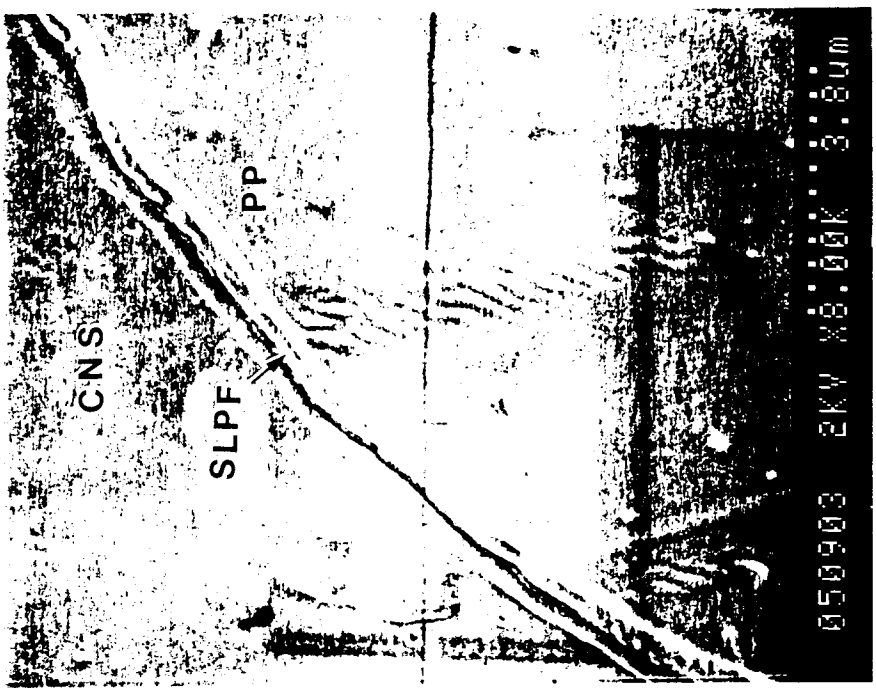


Figure 9


CNS
SLPF
PP
0509003 2KV X8.00K 3.8um


CNS
PP
0509001 2KV X8.00K 3.8um

Figure 10

Figure 11

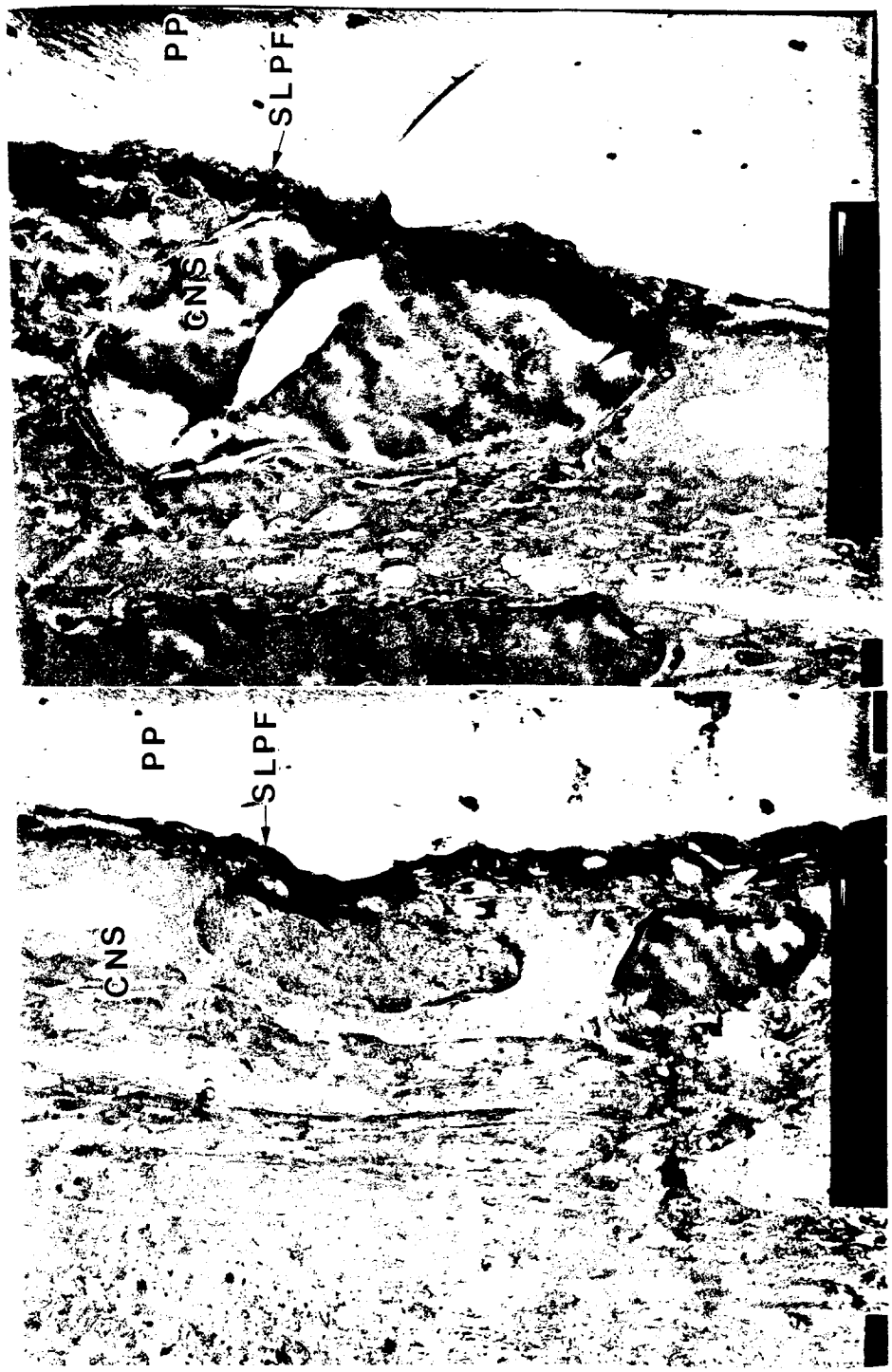


Figure 12.

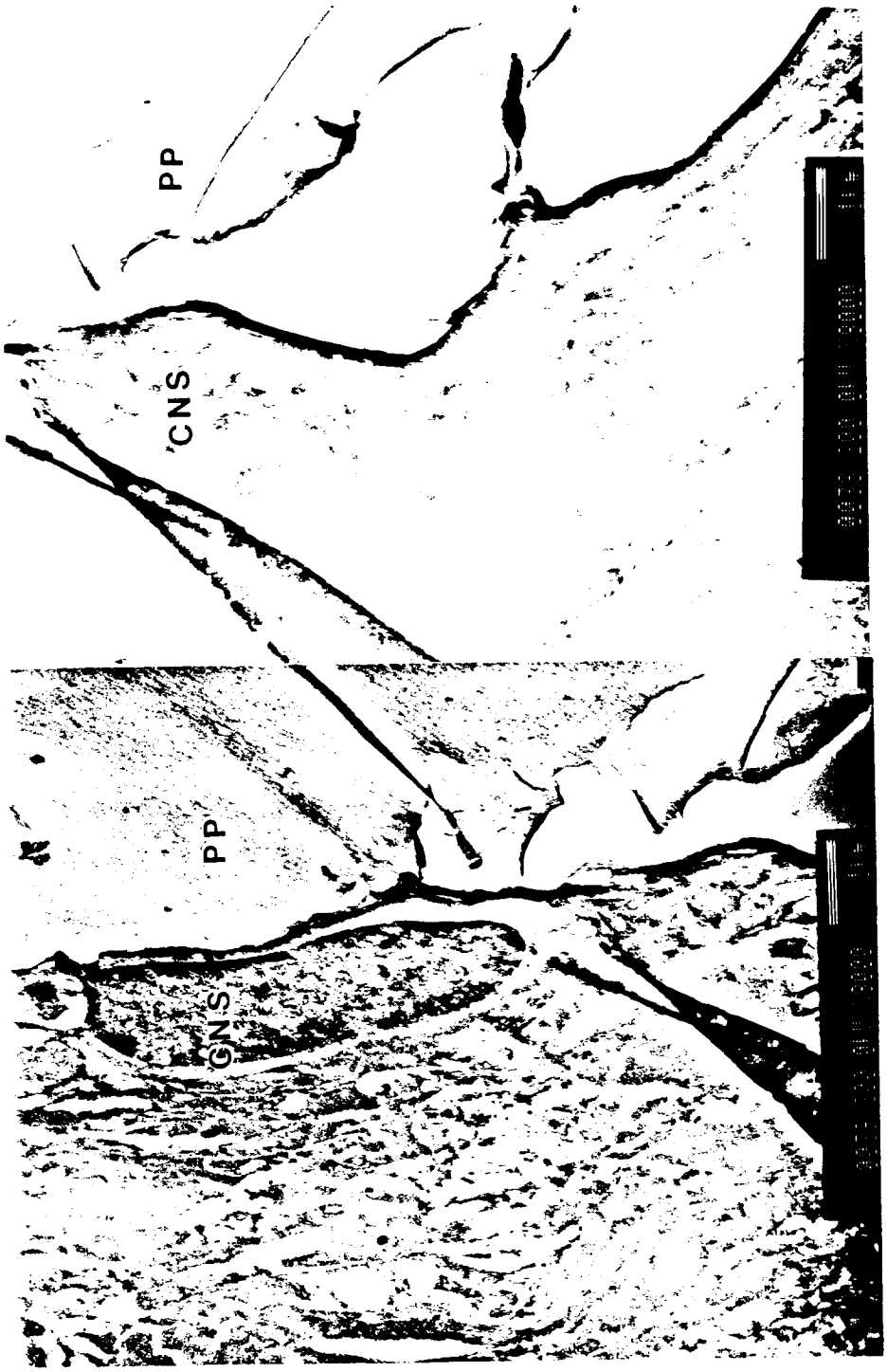


Figure 13